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Rat renal papilla: Comparison of two techniques for x-ray analysis

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Rat renal papilla: Comparison of two techniques for x-ray analysis. Major differences in elemental and water content in cells of rat papillae have been reported by investigators using the frozen hydrated/frozen dried method [4] versus that using external standards [1] for x-ray microanalysis. These differences could not be ascribed to either cryosectioning at warmer temperatures or to the analytical algorithm used by either group [7]. In this study, six paired left and right renal papillae were subjected to x-ray analysis. The frozen hydrated/frozen dried method was used on undipped papilla, while both methods were applied simultaneously to contralateral papillae dipped in albumin standard. No significant differences were seen between the physiologic state of the left and right kidneys prior to freezing. Our results demonstrated two major problems with application of an albumin peripheral standard: 1) albumin dipping significantly changed elemental and water content in papillary collecting duct cells, epithelial cells and interstitium, but interstitial cells were not affected; 2) the peripheral albumin standard itself also changed water and elemental content in a direction consistent with movement of Na and Cl from tissue to standard, and water from standard to tissue.

Application of x-ray microanalysis of Beck et al [1] using the Dorge, Rick, and Thurau method [2, 3] and by us [4] using the Saubermann et al method [5, 6] have reported major differences in the values for elemental and water contents of antidiuretic rat papillary compartments. The reasons for these reported differences are unknown. In an earlier study to determine if the differing microprobe techniques used in those reports could account for the differences measured, we analyzed elemental concentrations in rat proximal tubule cells using both methods simultaneously on the same cells. Both methods provided comparable results [7] and our method was able to determine accurately the elemental and water content of the albumin peripheral standard. Furthermore, no differences in elemental or water content were seen in proximal tubule cells cryosectioned with albumin at −53°C and those cryosectioned at −80°C. Thus the differing values reported by the two groups for elemental analysis of rat kidney could not be ascribed to either cryosectioning at a "warmer" temperature or to the analytical algorithm used by either group.

The renal cortex has a collagenous capsule separating the renal parenchyma from the isotonic albumin-Ringer's (Ringer's solution in 20% albumin) into which the kidney is dipped. The papilla is covered with a simple cuboidal transporting epithelium and has an axial osmotic gradient which makes it impossible to use an isotonic peripheral standard over the entire papilla. Further, the success of the external albumin method depends upon the validity of two major assumptions: the application of the peripheral albumin standard does not change the water or elemental content of the tissue to which it is applied; and that the elemental and water composition of the peripheral standard itself does not change. Since the quantitative algorithm requires application of a peripheral standard for determination of absolute values of elemental concentration and dry weight fraction, there is no independent means for testing those two assumptions. The frozen hydrated/frozen dried method, however, does not depend upon a peripherally-applied standard for absolute quantitation and one can, therefore, test those two assumptions inherent in the peripheral standard method. While results of analysis of cortex using both methods simultaneously demonstrated that both assumptions were valid for proximal tubule cells lying beneath the renal capsule, that might not be the case for papillae in which the albumin layer lies directly on the surface of transporting epithelia. Consequently, if those two assumptions were not valid for renal papilla, one might be able to offer an explanation for the differences between the results reported by the two groups. Therefore, to determine if the application of a peripheral albumin standard affects the elemental and water content of papilla, and to determine if that albumin peripheral standard remains unchanged, we undertook simultaneous analysis of paired antidiuretic rat papillae prepared according to Beck et al. [1]. In these experiments, after carefully documenting the physiological state of each kidney, one papilla was dipped in an peripheral standard containing albumin prior to freezing while the contralateral papilla was frozen directly without dipping in the albumin electrolyte peripheral standard. The frozen hydrated/frozen dried method for x-ray analysis of frozen hydrated tissue sections was used for analysis of both papillae and for the albumin standard, while the external standard method could then be used for analysis of the dipped papilla and not the standard.

Methods

Sample preparation

161 to 220 g male Sprague-Dawley rats were prepared according to the methods described by Beck et al [1]. To achieve antidiuresis, the animals were deprived of food and water

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overnight (14 to 16 hours). The animals were anesthetized with Inactin (100 mg/kg intraperitoneally). Body temperature was maintained at 37°C by a heated operating table. The trachea was exposed and cannulated, and polyethylene catheters were placed in the left femoral artery for blood pressure monitoring and into a jugular vein for isotonic fluid infusion. The left and right kidneys were exposed through a midline incision and freed of adherent fat and connective tissue. The left kidney was placed in a plastic cup and continuously bathed with warm mineral oil at 38°C. The left ureter was cannulated and the distal end of the ureter was tied off. The bladder was cannulated for collection of urine from the right kidney. After a two hour equilibration period, urine was collected under oil from each kidney for 30 min. The urine from each was then immediately analyzed for sodium, potassium, and osmolality. Based upon the analysis of the urine collected from the left kidney, a 20g% albumin solution with sodium and potassium concentrations and an osmolality similar to that of the sampled urine was then prepared by adding sodium acetate, potassium chloride, and urea to the albumin solution. Analysis of the urine and preparation of the albumin solution was kept to under 10 minutes.

To freeze the papillae, one person lifted the kidney with forceps and clamped the renal pedicle with a hemostat adjacent to the kidney. A second person simultaneously clamped the renal pedicle nearer to the midline and cut between the two hemostats. The order of removal of left and right kidney and the order in which the kidney was dipped in the albumin solution were randomly altered. After removal, the papilla was exposed, and a piece placed on a sharpened and forked wooden applicator stick and either plunged directly into melting Freon 22 (which was being stirred by a third person) or dipped into the albumin standard and then snap-frozen in the melting Freon. Removal of the kidney and freezing invariably took less than 12 seconds.

Papillae were then mounted in our cryochamber (Cryocut I, Burlington Scientific, North Billerica, Massachusetts, USA) which was mounted on a Sorvall MT2B microtome. Without pre-trimming the block, cryosections (0.5 μm nominal thickness) were cut at temperatures between −50°C to −55°C (average temperature −53°C) measured in the chamber atmosphere adjacent to the blockface. This cutting temperature was chosen to insure a flat, combined section of albumin and papilla with minimum discontinuous chip formation. A tungsten-carbide knife with a total cutting angle of 30° was used with a glass antiroll plate as described earlier [4–6]. Sections were then placed on a pre-cooled beryllium (Be) holder and grid which had been coated with nylon film. The cryosections were stored covered, in a special chamber within the cryomicrotome chamber prior to transfer. The frozen hydrated sections were then transferred from the cryomicrotome through an airlock into an AMRay 1400T scanning electron microscope, fitted with a specially designed cold stage (Burlington Scientific) cooled to −185°C. The transfer device consisted of a sealable Delrin cylinder which was evacuated by a rotary vacuum pump, immediately after the Be holder with the frozen hydrated sections was withdrawn into the chamber.

Analytical Protocol

Similar analytical conditions were used for all analyses: accelerating voltage 20 KeV, probe current (as measured by a specimen level Faraday cup) 0.4 nA, counting time 100 seconds (live time). Two analytical protocols were used on the albumin dipped papilla: 1) method reported by Saubermann et al [4–6], and 2) method reported by Beck et al [1].

The analytical algorithm used by us has been described in detail [5, 6]. Briefly, this consists of applying the ratio of characteristic counts to continuum counts corrected for extraneous sources estimated by continuum generation rate from the adjacent support film [8]. The characteristic count rates are determined from the area under the characteristic peak for an element after subjecting the spectrum to background suppression using a top hat digital filter technique. Continuum counts, as a measure of specimen mass, are obtained from the region between 4.6 and 6.0 KeV. The ratio obtained from the characteristic counts to background are then standardized using a standard curve for each element. In this case, no added correction for absorption of sodium x-rays was made. The formulas used for these calculations have been reported elsewhere [5, 6]. Our analytical protocol required analyses of continuum count rates carried out first in the hydrated state over large areas. By dividing these larger areas into smaller analytical areas, it can be shown that the larger area is of uniform mass thickness [5, 6]. The section was then freeze dried within the microscope vacuum by raising the stage temperature to −60°C for 30 min. The stage was then re-cooled to −185°C and the section re-analyzed in the dried state. Water content was calculated by comparing the "dried" continuum generation rate with the "wet" generation rate. Wet weight concentration (mmole/kg) was calculated from the dry weight concentration [4–6].

Using the Dorge, Rick, and Thurau method, analyses were carried out in the dried state. With their method, count rates of characteristic radiation from tissue were compared to characteristic counts from standard. In this study, these data were obtained from the filtered spectra as acquired above. Since the albumin layer served as the reference standard, it was not possible to determine its elemental content using this method.

Both the methods were applied to the albumin dipped papilla, while our method alone was applied to the non-dipped contralateral papilla. Since the intensities of characteristic x-ray counts (obtained after digital filtering) were suitable for application of both methods, the results reported here for the albumin-dipped papilla represent two different calculation algorithms using the same data.

Analyses were performed on the peripheral albumin standard on the dipped papilla; in addition collecting duct cells (CDC), papillary epithelial cells (PEC), interstitial cells (IC), and interstitium (I) were analyzed.

Results

Analyses were performed on pairs of renal papilla from four animals, and in addition, from the albumin-dipped papilla only from two additional animals. Physiological parameters including blood pressure are listed in Table 1. No significant physiological differences were detected between the left or right kidneys either by paired T-test or by unpaired T-test. Urine flow, Na, K, and osmolality were similar to that reported by Beck et al [1]. Mean urine osmolality for the left kidney was 1690 ± 194 (SEM) and was not significantly different when compared to the right kidney 1573 ± 184.

Table 1. Renal papilla—electron microprobe analysis (physiological parameters)

	UV *μl/min*	UV *μl/min/100 g*	U Na *mM/L*	U K *mM/L*	Uosm *mOsm/KgH₂O*	B WT *gm*	BP *mm Hg*	Alb.osm *mOsm/KgH₂O*
Left kidney								
Animal								
1	0.91	0.57	20	150	1613	161	100	1660
2	1.83	1.05	40	130	1700	174	120	1740
3	1.67	0.76	50	360	2490	220	130	2216
4	1.20	0.60	40	40	1084	200	125	1115
5	1.20	0.66	30	80	1394	181	115	1340
6	1.17	0.96	30	155	1859	178	115	1945
Mean	1.42	0.77	35	153	1690	186	118	1669
SEM	±.15	±0.08	±4.3	±45	±194	±8.6	±4.2	±163
Right kidney								
Animal								
1	1.50	0.93	50	90	1168			
2	1.83	1.05	60	150	1690			
3	2.17	0.98	40	450	2314			
4	1.40	0.70	20	10	1252			
5	1.09	0.60	20	60	1205			
6	1.66	0.93	10	135	1806			
Mean	1.61	0.87	33.3	149	1573			
SEM	±.15	0.07	±8.0	±63	±184			
Unpaired T-test								
T value	0.8837	0.9118	0.1832	0.0427	0.4390			
P NS	NS	NS	NS	NS	NS			
df 10								

Morphological detail was similar in both dipped and undipped sections. Albumin-dipped sections had albumin on their surfaces but also frequently had albumin visible within collecting duct lumens (Fig. 1). Digital x-ray imaging demonstrated the presence of sulfur within collecting duct lumens in these cases, which was not present in the non-dipped contralateral papillae.

The elemental distribution and water content of the pooled measurements of CDC, PEC, IC, and I, for both alumin-dipped and undipped papilla are shown in Table 2, as measured by our method. A significant increase in CDC water content in dipped papilla (75.0 ± 0.7% SEM) was observed when compared to non-dipped papilla (68.3 ± 1.0%). Water content of the other three compartments showed no significant difference between the albumin-undipped papilla and the albumin-dipped papilla. Na and Cl (mmole/kg wet weight) in dipped papilla were decreased when compared in CDC (Na: 124 ± 12 vs. 227 ± 16; Cl: 154 ± 13 vs. 269 ± 17), PEC (Na: 151 ± 11 vs. 254 ± 31; Cl: 186 ± 11 vs. 278 ± 32), I (Na: 518 ± 15 vs. 654 ± 31; Cl: 517 ± 15 vs. 652 ± 32). No difference was seen in elemental distribution or water content between undipped or dipped interstitium except for the S content which was significantly higher in the dipped papillae. K (mmole/kg wet weight) was decreased in dipped CDC (124.7 ± 4.4) compared to undipped CDC (154.1 ± 7.0); otherwise, no difference in K content was observed in the other compartments. P and S in the dipped CDC were both significantly decreased compared to undipped CDC.

Comparison of paired left and right kidneys showed similar patterns of elemental and water content and similar statistical differences when analyzed by paired Student's *t* test. The one exception to this was one set of paired samples where cryosections were obtained from a much more proximal location (hence, from an area having a relatively lower osmolarity) from the undipped papilla than they were from the dipped. In that case, analysis from the undipped papilla were not significantly different from that for the dipped papilla. In two cases of our six paired samples, we were unable to obtain analyses from both left and right papilla due to technical problems, which included a vacuum failure and a papilla which fractured during mounting in the microtome cryochamber, thereby making it impossible to obtain cryosections. Both of these technical problems affected the undipped papilla.

Elemental analysis of the peripheral albumin standard using our method is shown in Table 3 along with the original composition of albumin standard as measured by flame photometry; measured osmolality is given in Table 1. Dry weight content of the albumin standard was calculated to be 20 g/100 g (20%). X-ray analysis of the peripheral standard showed Na, K, Cl, and water contents which were different from that which was prepared. In general, Na and Cl content of the albumin standard was greater than expected (and measured by flame photometry) and water content was less than expected (with the exception of animal #1).

Calculations of elemental and dry weight content using the Dorge, Rick, and Thurau method are reported in Table 4. Two sets of calculations were made: method I used the assumed correct original concentrations for Na, Cl, K and dry weight within the peripheral albumin standard (Table 3) which, as explained above, differed from the measured values; method II used the concentrations for the elemental and water content of the peripheral albumin standard (Table 3) measured by x-ray microprobe analysis. As expected due to the changes in the albumin, major differences were observed between the respective values calculated by these two methods. In general, values calculated by method I were close to those values reported by Beck et al [1]; values calculated by method II were close to values obtained by our method.

Discussion

Two different techniques have shown major differences in the elemental and water content of rat papilla using two different

Fig. 1. *Scanning transmission image of a cryosection of rat papilla dipped in albumin electrolyte peripheral standard and freeze dried within the microscope vacuum at −60°C.* Note presence of dense material within some CD lumens. This dense material was similar in appearance and dry weight sulfur content to peripheral albumin standard. This dense material was not present in CD lumens of contralateral undipped papilla. (×760)

x-ray microprobe techniques. Since knowledge of elemental distribution and water content in antidiuretic papilla is important for understanding the physiology of the papilla concentration mechanism, it is of considerable importance to our understanding of renal physiology to determine why the measurements are so different and which measurements reflect the in vivo situation. In a previously reported study to ascertain if there were methodological problems inherent in either the analytical algorithm or analytical preparation of our two methods, we analyzed proximal tubule cells using both methods simultaneously [7]. Since our method uses frozen hydrated sections and does not require an adherent peripheral albumin standard for determination of absolute elemental concentrations and water content, we are able to provide validation of our method and the results reported by Beck et al [9] when used on renal cortex [7]. The results of those studies very clearly demonstrated that both our reported methodology for x-ray analysis of frozen hydrated tissue sections and x-ray microprobe analysis using the peripheral standard method for frozen dried sections can give comparable results with similar accuracy and precision [7]. Further, we established that there is no difference between the elemental distribution in sections cut at warm (−53°C) temperatures, and those cut at −80°C [7]. Therefore, other experimental factors must be considered to explain the differences reported for elemental distribution and water content in compartments of antidiuretic papilla by our two groups.

To apply the peripheral standard method requires that the tissue be dipped into the albumin-Ringer's solution which is thus frozen along with the tissue as an outer layer. Both the tissue and standard are cryosectioned simultaneously. After freeze drying, the adherent standard provides a means for absolute measurement of dry weight fraction. Two assumptions about this method, stated by Dorge et al [2], are that the cryosection of adherent standard and the tissue is of uniform thickness, and secondly, that during drying there is no differential shrinkage of the albumin standard or the tissue. Our previous study helped provide confirmatory evidence that these two assumptions are valid [7].

However, there are two additional assumptions which are inherent in the use of adherent peripheral standards. These assumptions, which have not been generally stated, but are crucial for the valid application of that method. They are: 1) the peripheral standard into which the tissue is dipped does not alter the distribution of elements and water in the tissue; and 2) the elemental distribution and water content of peripheral standard surrounding and adherent to the tissue remains identical to the original composition of the standard solution used for dipping—i.e., the standard does not change. The peripheral standard technique [1–3, 9], has no inherent way of testing those two assumptions since the quantitative method depends upon the use of values obtained from the peripheral standard. Since the frozen hydrated/frozen dried method does not depend upon a surrounding peripheral standard, it is possible to test the hypotheses that the peripheral standard does not change in composition and that the peripheral standard does not affect the elemental distribution of the tissue to which it is applied. Such assumptions appeared to be valid for proximal tubule cells and the cortex, perhaps due to the presence of a collagenous capsule separating the parenchyma from the peripheral standard.

In these experiments, one papilla was removed, frozen, cryosectioned and analyzed using our method of x-ray analysis, while the contralateral kidney was removed and dipped in a peripheral albumin standard and analyzed using both methods simultaneously. In this way, since both left and right kidney were physiologically in similar states (Table 1), one kidney served as a control for the other. Because the frozen hydrated/frozen dried method can determine the composition of the external albumin standard directly, one can ascertain if it is the same as the original standard.

The results of the experiments reported here clearly demonstrate that the application of a peripheral standard to the renal papilla altered the elemental distribution and water content in collecting duct cells, papillary epithelial cells, and interstitium, but in our experiment did not affect interstitial cells in the rat antidiuretic papilla. In addition, the Na, Cl, K concentrations and dry weight fractions of the peripheral standard was invari-

Table 2. Comparison of water and elemental content of albumin dipped and contralateral undipped papilla of antidiuretic rats[a]

	N	H_2O, %	Na	Cl	K	P	S
			mmole/kg wet weight				
Collecting Duct Cells							
Undipped	123	68.3 ± 1.0	227 ± 16	269 ± 17	154.1 ± 7.0	433 ± 17	33.9 ± 1.4
Dipped	140	75.0 ± .7[b]	124 ± 12[b]	154 ± 13[b]	124.7 ± 4.4[b]	363 ± 12[b]	28.6 ± 1.1[b]
Epithelial Cells							
Undipped	27	67.8 ± 2.3	254 ± 31	278 ± 32	131.8 ± 12.4	433 ± 41	40.1 ± 3.8
Dipped	88	69.6 ± 1.0	151 ± 11[b]	186 ± 11[c]	140.5 ± 5.7	403 ± 16	38.6 ± 2.0
Interstitial Cells							
Undipped	54	68.5 ± 1.5	362 ± 28	401 ± 31	137.0 ± 7.9	412 ± 27	22.7 ± 2.0
Dipped	103	66.3 ± .8	391 ± 23	429 ± 24	131.7 ± 5.7	403 ± 18	21.6 ± 1.5
Interstitium							
Undipped	78	83.1 ± .8	654 ± 31	652 ± 32	38.0 ± 2.0	29.8 ± 2.5	2.1 ± .4
Dipped	119	82.4 ± .6	518 ± 15[b]	517 ± 15[b]	40.5 ± 3.3	39.2 ± 4.9	8.6 ± 1.0[b]

[a] Data presented as mean ± SEM; N represents number of measurements.
[b] $P < 0.005$
[c] $P < 0.01$

Table 3. Composition of albumin electrolyte peripheral standard

	Original composition of albumin standard *mmole/L*		Elemental and water content of albumin electrolyte peripheral standard measured with x-ray analysis *mmole/kg*			
Animal	Na	KCl	Na	Cl	K	% H_2O
1	40	147	87.4 ± 7.2	96.8 ± 7.6	46.1 ± 1.6	83.7 ± 1.5
2	56	123	230 ± 49	345 ± 55	66.1 ± 9.9	79.6 ± 2.6
3	65	347	84.6 ± 11.2	335 ± 38	350 ± 41	72.5 ± 1.8
4	61	44	139 ± 37	144.6 ± 30	77.8 ± 10.2	78.2 ± 2.6
5	39	77	86.5 ± 8.0	129.7 ± 12.3	142.7 ± 16.1	75.0 ± 2.2
6	52	144	179.1 ± 8.9	177.8 ± 18.8	163.5 ± 20.1	75.1 ± 2.7

ably different from that which we prepared and assayed via flame photometry (Table 4).

In our previous study, we were able to show that we could measure with reasonable accuracy and precision the elemental and water content of the albumin peripheral standard when applied to proximal tubule cells. In this study, we observe great variation in albumin Na, Cl, and K content over that which was prepared. In general, our results showed an increase in Na and Cl in the albumin, while tissue compartments showed a decrease in Na and Cl compared to that measured in the non-dipped papilla. The variability we observed is not unexpected since the cryosections of papillae were taken at a random depth from the tip, although they were all taken from a sample within the first two mm. The albumin-Ringer's standard was made so as to be isotonic with the ureteral urine collected over a 30 min period from the left kidney of each animal. However, the final osmolarity is probably not isotonic with regions of papilla proximal to the tip, or for that matter at the tip, due to changes in the renal pelvis [10]; hence, it is reasonable to expect that osmotic gradient between the albumin standard and the papilla to increase as a function of the proximal distance from the papilla tip. Such a gradient might be expected to add to the probability of changing concentrations in the albumin-Ringer's standard. In addition, the time between application of standard and freezing would also be expected to alter the magnitude of elemental changes in the peripheral standard. Therefore, in this case there would be no consistent way to predict the actual content of the peripheral standards, since diffusion or transport could be greater or lesser depending upon position, elemental and osmotic gradient magnitude, and time.

Albumin standard was found within collecting duct lumens, and in that location had a different concentration of elements and water than the peripheral albumin standard. The peripheral albumin standard was generally much thinner than that which adhered to renal cortex, and our measurements of the standard were based upon the small amounts which remained adherent. In the paper by Beck et al [1], they published an example of their dried cryosection of papilla. Our albumin dipped sections were similar in appearance. Although the lumen of our specimens usually contained more albumin standard than that seen in the micrograph of Beck et al [1], the amount of albumin present peripherally was similar. All x-ray measurements of albumin standard contained P of varying amounts. Since no phosphorus should be present in the standard we are forced to conclude that the P in the peripheral standard method came from the papillae (presumably from urinary phosphates). The presence of P also indicates that there was sufficient time for some mixing to occur between urine and albumin standard. If the composition of the urine along the surface of the more proximal papilla was not the same as the urine composition of the urine we collected from the ureter [10], it would be reasonable to expect such a difference to contribute further to the variability we observed in measurement of the albumin standard. Such a supposition is consistent with the theories proposed by Bargman et al [11].

Table 4. Comparison of calculations of papillary dry weight and elemental composition[a]

	Values reported by Beck et al [1]	Current Study			
		Beck Method		Saubermann Method	
		Calculation method I[b]	Calculation method II[c]	Albumin dipped	Undipped
Collecting duct cells					
% dry wt	19.7 ± 0.5	24.0 ± 0.8	26.9 ± 0.8	25.0[d]	31.7[d]
Na (mmole/kg wet wt)	27.5 ± 1.4	61.2 ± 4.1	137.4 ± 7.9	124 ± 12	227 ± 16
Cl (mmole/kg wet wt)	76.3 ± 2.5	123 ± 11	162.0 ± 8.0	154 ± 13	269 ± 17
K (mmole/kg wet wt)	135.2 ± 2.5	162 ± 10	144.7 ± 4.8	124.7 ± 4.4	154 ± 7
Papillary epithelial cells					
% dry wt	20.9 ± 0.9	28.9 ± 1.2	33.5 ± 1.1	30.4[d]	32.2[d]
Na	22.5 ± 2.3	94.8 ± 5.7	198 ± 15	151 ± 11	254 ± 31
Cl	79.9 ± 3.6	192 ± 16	226 ± 15	186 ± 11	278 ± 32
K	135.9 ± 4.8	152 ± 18	171 ± 7.7	140.5 ± 5.7	132 ± 12
Interstitial cells					
% dry wt	21.0 ± 0.9	34.2 ± 1.4	39.8 ± 1.4	33.7[d]	31.5[d]
Na	50.2 ± 3.0	292 ± 19	550 ± 30	391 ± 23	362 ± 28
Cl	107.2 ± 4.3	565 ± 47	553 ± 29	429 ± 24	401 ± 31
K	138.9 ± 4.2	169 ± 16	170.8 ± 8.4	131.7 ± 5.7	137 ± 8
Interstitium					
% dry wt	11.8 ± 0.5	15.6 ± 0.6	18.5 ± 0.7	17.6[d]	16.9[d]
Na	437.2 ± 18.8	340 ± 12	686 ± 26	518 ± 15	654 ± 31
Cl	437.5 ± 19.9	550 ± 28	638 ± 25	517 ± 15	652 ± 32
K	34.9 ± 2.2	30.9 ± 2.5	41.2 ± 1.4	40.5 ± 3.3	38 ± 2

[a] All values reported ± SEM.
[b] Calculation based upon assumption that albumin peripheral standard remained unchanged from original concentrations.
[c] Calculation based upon x-ray microprobe measured values for elements and dry weight fraction in albumin electrolyte peripheral standard.
[d] Calculated from % water.

Further, the presence of albumin standard in CD lumens may also have had an effect on the papilla, either through direct mechanical blockage of collecting ducts or through means of transport or diffusion mechanisms.

The Dorge, Rick, and Thurau method, or any method which depends upon a peripheral standard, assumes that the standard's composition is known. In the case of application to cortex, that assumption was shown to be correct although Beck et al [9] had no means to substantiate that critical assumption. It was not correct for the papilla. By assuming that the x-ray intensities determined from the standard represented a known amount of element or dry weight fractions, it would lead to erroneous calculated concentrations in the papillarycompartments.

Physiologically, both left and right kidney were similar (Table 1) and agreed with values reported by Beck et al [1]. Therefore, the differences we observed in the distribution of water and elements between the dipped and undipped kidney cannot be ascribed to differences in concentrating state. The compartments which appeared to change the most were those compartments adjacent to the albumin layer (PEC and CDC). The changes we observed in the albumin dipped kidney were not, in themselves, sufficient to explain the difference in the results of our earlier reported values and those of Beck et al [1], although those observed changes were clearly in the direction of the differences between our two reports. However, the combination of the changes observed in the dipped papilla and the use of the peripheral albumin standard assumed to be unchanged are sufficient to explain, in principle, the reported differences between our two studies, with the noted exception of the values for the interstitial cells which did not appear to be altered by the dipping process in our studies (perhaps due to their distance from the albumin layer).

Values obtained by our method for the nondipped papilla reconfirmed the patterns of elemental and water content previously reported by us in non-physiologically monitored adult antidiuretic rats [4], although some difference would be expected due to differences in degree of urinary concentration. This present confirmation was carried out on a different instrument in a different laboratory. It is interesting that in these experiments we observed higher phosphorous levels than in our earlier reported study. It may be that these differences in P may be related to the effects of anesthesia or surgery over the two hours required for the experiment. In both of our studies, we observed interstitial cells with high Na and Cl. Beck et al, however, reported low interstitial cell Na and Cl which were similar to their values for CDC and PEC. We cannot explain that difference with the failure of the two assumptions of their method noted above. Given the magnitude of these differences in interstitial cell Na and Cl, it is possible that these differences may represent a biological or physiological difference. Beck et al [1] used very young rats in their study (50 to 120 g rats), while we used larger and hence, more mature rats. Thus, it is possible that this difference in interstitial cell Na and Cl is in part related to age. There are, however, other methodological differences which might be considered as possible causes for this difference. Beck et al transferred frozen dried cryosections to their microprobe under dry nitrogen. If there were partial rehydration, it is possible elemental transformation might occur, and coupled with the fallacious assumption that the albumin standard is unchanged, may account for such differences. Alternatively, there might be increased susceptibility of inter-

stitial cells to radiation damage which would be more obvious with the Dorge, Rick, and Thurau method, than with our method, since their analysis is carried out without a cold stage at warmer temperatures. Another possible explanation is that the interstitial cells, located further from the albumin, may require longer for their elemental distribution to change. Thus a small delay prior to freezing may have a significant effect. It is also of interest that their reported values for Na and Cl are always different from each other in the cortex and in the papilla. Some of this can be explained by the effects of dipping on the papilla (Table 2) and the altered standard. In their method, cryosections are sandwiched between two formvar films. Such a specimen would be more susceptible to Na x-ray absorption, which may explain the lower values for Na when compared to Cl in their studies. Clearly, additional experiments are necessary to reconcile fully their results with ours.

It is interesting that structural elements (P and S) were not generally effected by albumin dipping. The difference observed in P and S in CD are due to the increased water content but Na, K, and Cl are changed (as determined from comparison of dry wt). Interstitium S was increased in dipped papilla but not phosphorus. Since albumin contains S, we cannot help but wonder if albumin or S somehow entered the papilla interstitium.

Due to the probability of sampling the papilla at different levels during cryosectioning (different levels along its axial osmotic gradient) and therefore, sampling differences in concentrations, variance is to be expected. In fact, the observed variance exceeded the variance we observed in other tissue [12] and in cortex [7] but was similar to that which we noted in our previous report of elemental and water content of papilla [4]. Comparing individual pairs of kidneys show similar changes to those reported for pooled samples, although the magnitude of the changes was variable. Since cryosectioning of each kidney represents a similar random sampling of a similar zone for papilla, using a pooled sample approach seems reasonable. Individual pairs of dipped and undipped kidneys each represented an independent sample of papilla at levels from the tip which were probably not at exactly the same level and hence did not represent a paired sample of the same sample depth.

A number of investigators have applied the peripheral standard technique or variations of it [13–15]. Gupta and Hall, who have developed a method for frozen hydrated section analysis using a peripheral standard after the methods of Dorge, Rick, and Thurau [17], have reported recently, in a paper by Dow et al [16], on the distributions of elements in the posterior midgut of the tobacco hornworm *(Manduca sexta)*. It is interesting that in that study, they looked at a sample unexposed to a peripheral standard (in vivo sample) as well as samples with adherent peripheral standard (in vitro). They noted differences in elemental and water content between the in vivo sample and the in vitro samples. In the in vivo sample, K gradients were better maintained than the in vivo sample [16]. Such differences may be indicative of the same problems with use of peripheral standards reported here, and points to the importance of adequate controls.

In conclusion, the reported differences in papillary elemental distribution and water content can be largely explained by application of two untested assumptions which may not be warranted in all cases, inherent in the external standard method [1, 4]. Our present results reconfirm our earlier results that interstitial cells contain large amounts of Na and Cl.

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